Reference: *Biol. Bull.*, **155**: 317–335. (October, 1978)

GUT ULTRASTRUCTURE AND DIGESTIVE PHYSIOLOGY OF TWO MARINE NEMATODES, *CHROMADORINA GERMANICA* (BÜTSCHLI, 1874) AND *DIPLOLAIMELLA* SP.[1]

ALICE DEUTSCH[2]

Department of Biology, City College of New York, New York, New York 10031

Relative to other phyla, the Nematoda encompasses large numbers of species living in virtually every known benthic habitat. There have been several approaches to understanding the apparent coexistence of large numbers of nematode species. In studies of field populations, variables such as temperature, oxygen concentration, and salinity have been related to the abundance and distribution of nematode species (*e.g.*, review by McIntyre, 1969; Pamatmat, 1968; Tietjen, 1969, 1971; Coull, 1970; Meyers, Hopper, and Cefalu, 1970; Warwick and Buchanan, 1970; Warwick, 1971; Ward, 1973; Wieser and Schiemer, 1977). Other approaches have used a laboratory setting to determine nematode requirements for or tolerances to environmental variables (*e.g.*, Tietjen, Lee, Rullman, Greengart, and Trompeter, 1970; Gerlach and Schrage, 1971; 1972; Tietjen and Lee, 1972; Hopper, Fell, and Cefalu, 1973; Ott and Schiemer, 1973; Schiemer, and Duncan, 1974).

In shallow benthic environments, a large array of potential foods for nematodes exist (Lee, McEnery, Kennedy, and Rubin, 1975; Fenchel, 1969). The heterogeneous distributions of different food species may account for much of the variety and large numbers of nematode species found in the benthos, assuming that different nematode species are feeding on different foods. Wieser (1953, 1960) theorized about the likelihood of nematodes being feeding specialists—living in similar microenvironments but existing on different foods in the benthos. He grouped nematodes into feeding types according to the size of the buccal cavity and the extent and type of buccal armature. He reasoned that differences in nematode diets could be due to different mouthparts and, therefore, to differing abilities to ingest various food species. Recently, tracer feeding techniques have been used to demonstrate that selective feeding may occur in several nematodes (Tietjen, Lee, Rullman, Greengart, and Trompeter, 1970; Tietjen and Lee, 1973, 1977).

Seemingly slight differences in the digestive physiologies of marine nematode species may reflect adaptations to different diets. It is generally assumed that nematode digestion, aside from the process of ingestion, conforms to a similar pattern throughout the taxon—free-living soil, freshwater, and marine, as well as parasitic forms (reviewed by Lee, 1965; Bird, 1971; Lee and Atkinson 1976). The digestive tract is a straight tube differentiated into buccal cavity, esophagus, esophageal-intestinal valve, intestine, and rectum. There are three esophageal

[1] This paper is based on a thesis submitted in partial fulfillment of the requirements for the Ph.D. degree, City University of New York.

[2] Present address: Department of Anatomy and Cell Biology, State University of New York, Downstate Medical Center, 450 Clarkson Avenue, Brooklyn, New York 11203.

gland cells that empty their contents into the esophageal lumen. The esophagus is usually a pumping organ which forces food into the intestine. The intestine consists of a single layer of cells. Digestion is extracellular in nearly all species, although an intracellular phase does occur in some (Colam, 1971a, b).

The purpose of this study was to compare the digestive physiologies of two species of marine nematodes, including the microanatomy of their digestive tracts, and to compare their abilities to ingest and digest different foods. Ultimately, this information might help to explain the large numbers of nematode species coexisting in the benthos.

Materials and Methods

Nematode culturing

Two nematode species, originally isolated from a salt marsh at Towd Point, Southampton, Long Island, New York, were cultured for this study: *Diplolaimella* sp. and *Chromadorina germanica* Bütschli 1874. Nematodes were maintained in 60 mm plastic petri dishes with an overlay of 10 ml of 0.45 μm HA millipore-filtered sea water (mfsw) with a salinity of 26‰. A mixture of an antimycotic/antibiotic (penicillin 100 U/ml, fungizone 0.25 mcg/ml, streptomycin 100 mcg/ml) was added to the medium. The cultures were maintained at 20°–24° C under a light regime of 18 L : 6 D. Approximately 0.1 ml of suspended food organisms, at a concentration of 10^8 cells/ml, were added to each dish. These foods were obtained from stock cultures of algae grown on "S" medium (Lee, Stone, Muller, Rullman, and McEnery, 1970). The seawater overlay was changed once a week. Nematode cultures were transferred about once a month. In addition to the added foods, other species of algae and bacteria grew in the culture dishes, and thus cultures were considered to be agnotobiotic.

Electron microscopy

Whole specimens of *Diplolaimella* sp. were fixed for 2 hr at 4° C in 3% glutaraldehyde in millipore-filtered seawater (mfsw). Specimens were rinsed briefly in mfsw and post-fixed in 2% osmium tetroxide in mfsw for 2 hr at 4° C. Whole specimens of *C. germanica* were fixed for 1 hr at 4° C in 3% glutaraldehyde in mfsw; but immediately after the fixative was added, the cuticle was cut open by cutting off the anterior end of the nematode. Specimens were then processed as above.

Specimens of both species were dehydrated with acetone using the sequence 30% acetone for 10 min at 4° C, 70% acetone overnight or up to two days at −11° C, 90% acetone for 15 min at 4° C, three changes of 100% acetone 15 min each at 20° C. Specimens were placed in a mixture of 1 : 1 acetone to Spurr's embedding medium (1969), placed under vacuum for 45 min, and left uncapped in the hood for an additional 45 min. Finally, specimens were embedded in fresh Spurr's and polymerized at 70° C for 24 hr.

Primary fixation was carried out in small glass wells which were in turn placed in petri dishes to prevent evaporation. Fixative was removed carefully by

Pasteur pipette and several drops of 1% agar in mfsw at 47° C were added to the well. This was immediately mixed with a metal spatula to suspend the nematodes in the agar matrix. When the agar had hardened, it was transferred to a glass vial filled with the appropriate rinse solution. Processing continued with nematodes suspended in this agar for all steps up to and including embedding. To embed, small chunks of agar containing specimens were cut out with a razor and forceps under a dissecting scope and placed in flat embedding trays.

Light gold to silver to gray sections were prepared with an LKB ultramicrotome using a diamond knife. Sections were mounted on uncoated 200-mesh or 300-mesh copper grids (Polysciences, Inc.), stained with saturated aqueous uranyl acetate followed by 3% lead stain (Sato 1967), and viewed with a Philips 300 Electron Microscope at 80 kv. Kodak Electron Image plates were used for taking pictures.

Feeding experiments

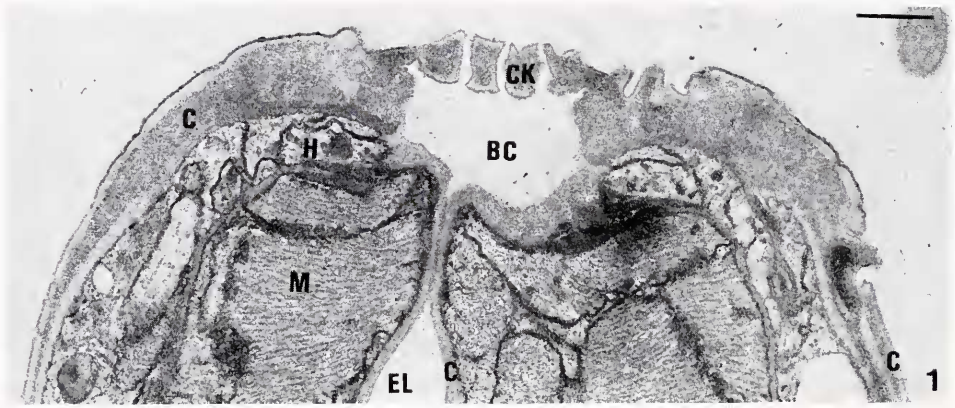
For this series of experiments, nematodes were fed a variety of different potential food species to test nematode ability to ingest and digest each food. About 30 to 50 adult nematodes were gently pipetted into sterile petri dishes, taking care to transfer very little of the medium into the new dish. Individual microfloral species were harvested from stock cultures by centrifugation, washed and resuspended in 0.2 ml mfsw. The food cells chosen for these experiments included six algal and two bacterial species and they were fed to nematodes as single foods. For controls, nematodes were pipetted into dishes without food. Dishes were examined and counted daily for live nematodes for up to five days. Some nematodes were removed after one to two days and processed for the study of gut contents. Food cells were processed along with the nematodes.

Histochemistry and cytochemistry

Specimens were fixed in 3% formaldehyde in sea water for 2 hr. Specimens were washed briefly in mfsw and incubated for nonspecific esterase activity using the indoxyl acetate method of Holt (1958) and arylamidase activity (Burstone and Folk, 1956). Controls included specimens incubated without the substrate and after heat inactivation at 100° C for 5 min. Whole mounts were examined with the light microscope.

For visualization at the electron microscope level, nematodes were fixed in 1% glutaraldehyde in sea water for 15 min, washed with mfsw 1 min, incubated for acid phosphatase (Barka and Anderson, 1962) at room temperature for 15 min, rinsed in mfsw, and then fixed again in 3% glutaraldehyde in sea water for 30 min. Routine procedures were followed for post-fixation, dehydration, and embedding.

For visualization of surface proteins in the fibrillar matrix lining the luminal borders of the intestinal cells of *Diplolaimella* sp., specimens were fixed routinely and dehydrated and embedded without post-fixation. The test for glycoproteins was made on sections collected on uncoated grids and immersed for 5 min in 0.5% phosphotungstic acid in 0.1 N hydrochloric acid, pH 1.2 (Quintarelli, Bellocci, and Geremia, 1973).



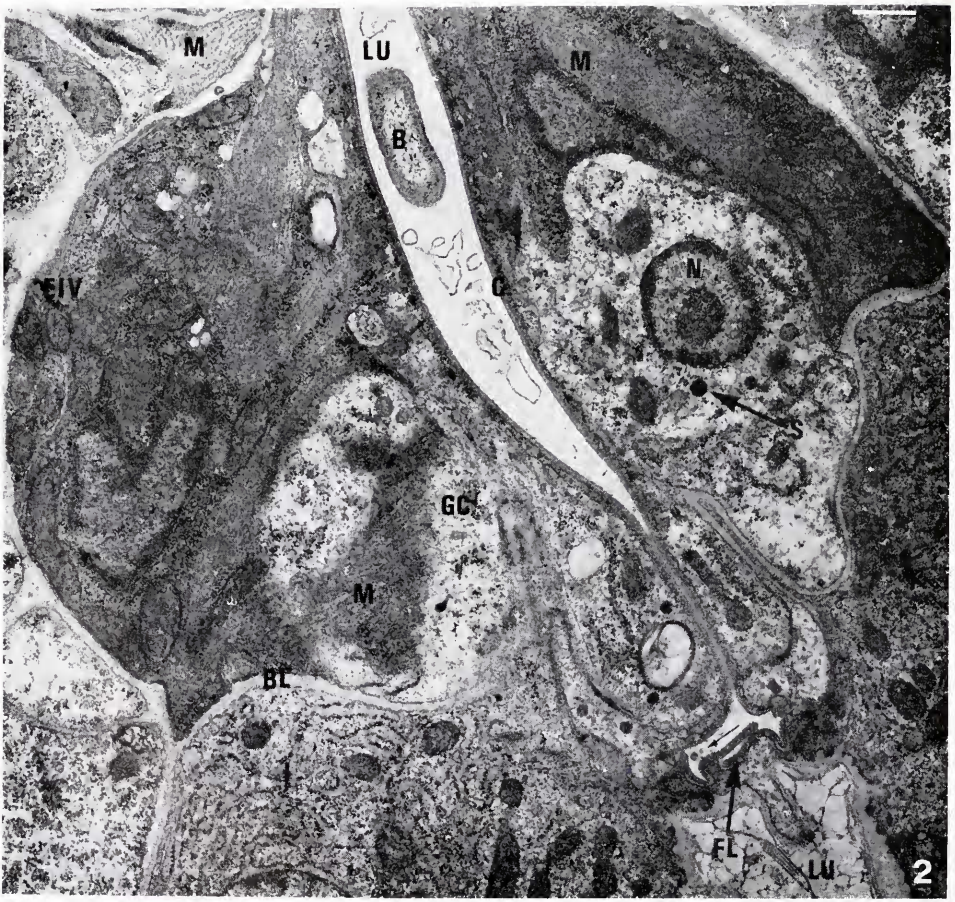


FIGURE 1. An obliquely longitudinal section through the anterior end of *Diplolaimella* sp. showing the buccal cavity and esophageal lumen lined with cuticle and transverse muscles. BC =

Results

Ultrastructure of the digestive tract: Diplolaimella sp.

Diplolaimella sp. has a typical nematode digestive tract, consisting of buccal cavity, muscular esophagus, esophageal-intestinal valve, intestine, and rectum. The buccal cavity is a circular opening approximately 1 μm in diameter surrounded by 12 rounded cuticular knobs (Fig. 1). It leads into the triradiate esophagus, which is cylindrical in shape anteriorly, but forms a bulb-like swelling at its posterior end. It is a typical nematode esophagus with no unusual features.

Between the esophagus and the beginning of the intestine is a cuticular-lined esophageal-intestinal valve consisting of several cells (Fig. 2). The valve is separated from the esophagus by a small band of encircling muscle filaments. The valve also has sets of muscle filaments which run radially from the cuticle lining the valve lumen to the external wall of the valve. Just posterior to the muscle cells are several secretory cells or gland-like cells which form the rest of the valve. These secretory cells protrude anteriorly into the valve muscle bands for part of their length. They contain membrane-bounded inclusions filled with fibrillar and electron-transparent materials or electron-dense materials. The muscles of the valve may, because of the position of these secretory cells, squeeze secretions out of the cells into the valve lumen when the muscles contract. No ducts were found, but the posterior valve lumen usually contained several dense granules than could have originated from these secretory cells.

The intestine is formed by a single layer of epithelial cells. In any cross-section only two cells circumscribe the intestinal lumen. These cells have very little stratification and exhibit cell organelles scattered at random throughout the cell.

The first two anterior sets of cells are typical secretory cells (Fig. 2). They each have a well-developed and extensive, rough, endoplasmic reticulum. There are several Golgi complexes. A few electron-dense cell inclusions are scattered in the distal regions of the cells. The microvilli are approximately 0.12 μm in diameter and 0.5 μm in length. They are closely packed at the cell surface bordering the intestinal lumen. Microfilaments are oriented along the longitudinal axes of the microvilli. A thin basal lamina bounds the intestinal cells at their external borders.

The remaining intestinal cells are all similar in structure and are not specialized along the length of the intestine (Fig. 3). Each cell has a well-developed, rough, endoplasmic reticulum and large numbers of mitochondria which are distributed throughout the cell. Occasionally, a multivesicular body occurs in these cells. Much of the cell volume is taken up by cell inclusions which have been described in the literature as "pigment granules." These are single membrane-bounded spheres

---

buccal cavity, C = cuticle, CK = cuticular knobs, EL = esophageal lumen, H = hypodermis, M = muscle. Bar is 0.5 μm.

FIGURE 2. Semi-longitudinal section of *Diplolaimella* sp. showing part of the muscle band that encircles the valve lumen, the valve's muscular bulb, and its gland–like cells. The anterior intestine is also visible. The secretory products are very electron dense. The small arrow points to secretory-like material in the valve lumen. The flaps separating the valve lumen from the intestinal lumen are also shown. B = bacterial cell, BL = basal lamina, C = cuticle, EIV = esophageal-intestinal valve, FL =flaps, GC = gland-like cell, I = intestine, LU = lumen, M = muscle, N = nucleus, S = secretory product. Bar is 0.5 μm.

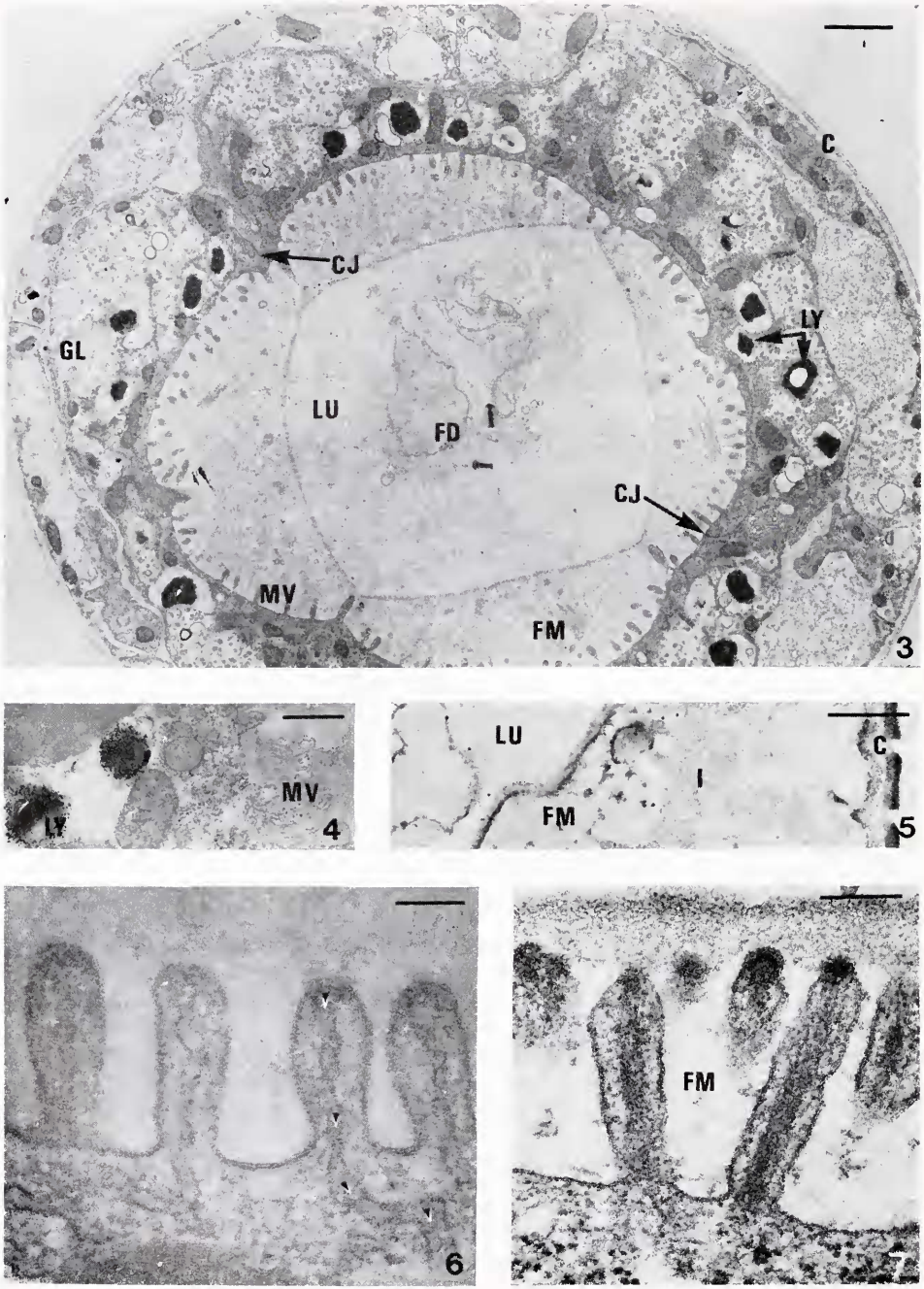


FIGURE 3. Typical transverse section through two intestinal cells of an adult female *Diplolaimella* sp. Unusual microvilli are at the four "corners" of the "square" outlined by the

containing very electron-dense materials. Some of these granules are positive for acid phosphatase, showing that some are actually lysosomes (Fig. 4). Less electron-dense granules are found peripheral to the lysosomes. They are not acid-phosphatase positive, but may represent an earlier stage in the production of the lysosomes. Because these intestinal cells lack any secretory apparatus, they are most probably absorptive cells.

The entire intestinal lumen is coated along the luminal surfaces of the cells with a well-organized fibrillar matrix (*e.g.*, Fig. 3). In transverse section it sometimes appears to be formed into four ridges that protrude into the lumen and four valleys or rays. Within the fibrillar matrix three zones of varying electron density could be distinguished. There is a narrow dense zone at the lumen-matrix interface which occasionally resembles a membrane but is not a typical unit membrane. There is a less dense homogeneously fibrillar matrix just under this zone. The third zone, closest to the intestinal cell membrane, occupies the largest volume of the fibrillar matrix. This zone of the matrix is fairly uniformly diffuse but within it are electron-dense fibrillar globules, parallel sheets of fibrils, and membrane-bounded vesicles (Fig. 3). The vesicles appear to contain the same fibrillar material which is found outside them. These vesicles are most numerous at the very beginning of the intestine, and sometimes occur in the matrix lining the rest of the intestinal cells. Their origin may be the anterior secretory cells of the intestine. The vesicles were sometimes found between the microvilli at their bases, throughout the inner matrix zone, and especially collected just beneath the middle zone. They may play a role in the maintenance and renewal of the fibrillar matrix.

The chemical composition of the matrix is unknown. There was staining of the narrow zone of the lumen-matrix interface, some small areas within the third zone of the fibrillar matrix, and the external cuticle with phosphotungstic acid applied to nonosmicated thin sections (Fig 5). It is possible, then, that the fibrillar matrix has proteins similar to those found in the cuticle.

The luminal surfaces of these intestinal cells are modified to form the very unusual microvilli (Fig. 6) which were sometimes found in the anterior four intestinal cells. Each microvillus had a cisterna along its central axis that was 0.02–0.04 μm in diameter and continuous with the endoplasmic reticulum. The cisterna ended blindly near the tip of the microvillus. The microvilli are regularly spaced but not as closely packed as in the anterior cells. In well-fed specimens, these

---

fibrillar matrix. C = cuticle, CJ = cell junction, FD = food debris, FM = fibrillar matrix, GL = glycogen, LU = lumen, LY = lysosome, MV = microvilli. Bar is 1 μm.

FIGURE 4. An oblique section through the intestine of *Diplolaimella* sp. showing lead deposits in the "pigment granules" from the acid phosphatase reaction, not counterstained. LY = lysosome, MV = microvilli. Bar is 0.5 μm.

FIGURE 5. Part of a transverse section through the intestine of *Diplolaimella* sp. that was stained with phosphotungstic acid at pH 1.2. Both the cuticle and luminal border of the fibrillar matrix show electron density (as does some food debris in the lumen) ; FM = fibrillar matrix, LU = lumen, I = intestine. Bar is 0.5 μm.

FIGURE 6. Detail of the microvilli of the intestinal cells of *Diplolaimella* sp. showing the cisterna of the microvillus continuous with the cisterna of the endoplasmic reticulum. The arrowheads trace this continuum. Bar is 0.1 μm.

FIGURE 7. Detail of the unusual microvilli associated with the fibrillar matrix of *Diplolaimella* sp. FM = fibrillar matrix. Bar is 0.2 μm.

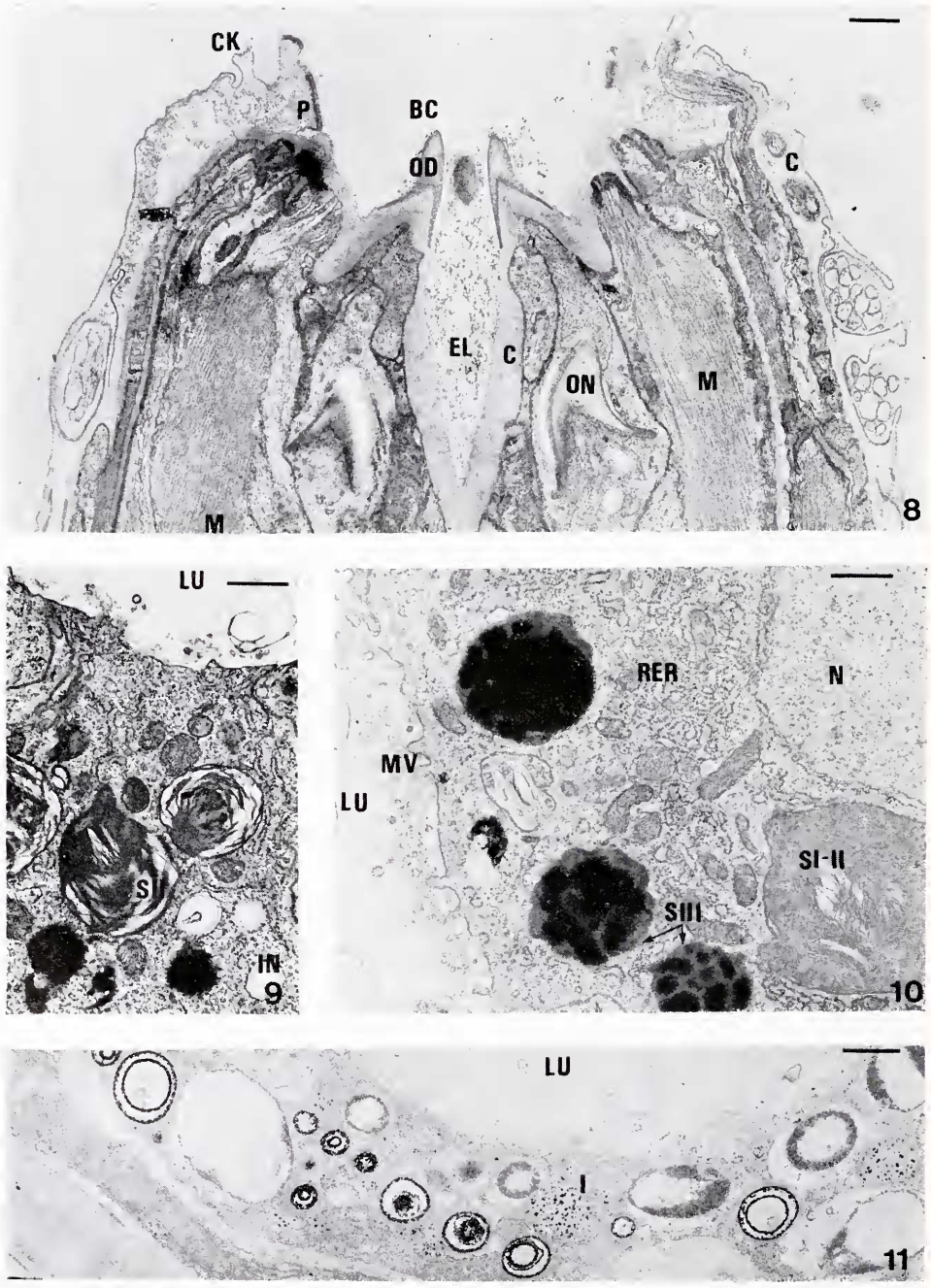


FIGURE 8. Longitudinal section through the anterior end of *C. germanica* showing the pads forming the mouth, the two sets of teeth (odontia and onchia), and longitudinal muscles. BC =

cells lose their usual appearance of a clearly defined cisterna along the central axis of each microvillus. Instead, the microvilli appear swollen and more electron-transparent.

There are specialized microvilli located immediately adjacent to the intercellular junction between the two circumluminal cells and in the area of the cell membrane equidistant between the two cell junctions. These four sets of longitudinally aligned microvilli are filled with microfilaments running along the central axis of each. The tips of these microvilli have a very electron-dense cap just under the plasma membrane of the microvillus (Fig. 7). Trailing from the tip of each specialized microvillus is dense fibrillar material which is continuous with the middle zone of the fibrillar matrix. The microfilaments in these microvilli may anchor and stabilize the middle zone of the matrix. These microvilli may function to maintain the physical integrity of the matrix.

Ultrastructure of the digestive tract: Chromadorina germanica

C. germanica has a larger mouth than *Diplolaimella* sp. (Fig. 8). The mouth measures approximately 2.9 μm in diameter. An unusual set of small pads, analogous to lips, surround the buccal cavity. Just lateral to the pads, the external cuticle is sculptured with knob-like projections. The pads, together with these knobs, may help to hold and position food particles for subsequent ingestion.

The entire buccal cavity is lined with a dense, unsculptured cuticle. The cuticle is modified to form three electron-dense sharp pointed teeth, or odontia, protruding from the base of the buccal cavity and surrounding the entrance to the esophagus (Fig. 8). Muscle fibers run posteriorly from their attachment points at the cuticle lining the buccal cavity to the esophageal musculature. These longitudinally-running muscle fibers may help to temporarily enlarge the buccal cavity and expose the teeth. This contrasts with *Diplolaimella* sp., which has neither pads nor odontia and where all muscles around the buccal cavity run transversally.

The buccal cavity opens into the esophagus just posterior to the three teeth. Another set of three teeth, or onchia, which are pointed elaborations of cuticle, are embedded in the anterior end of the esophagus (Fig. 8).

While the esophagus is generally similar to that of *Diplolaimella* sp., it differs from the latter in several respects. The dorsal esophageal gland cell empties into the anterior portion of the esophageal lumen somewhat posterior to that of *Diplolaimella* sp. and the bulb of the esophagus of *C. germanica* is larger and wider than that of *Diplolaimella* sp. In contrast with the latter, each muscle filament is

---

buccal cavity, C = cuticle, CK = cuticular knob, EL = esophageal lumen, M = muscle, OD = odontia, ON = onchia, P = pad. Bar is 0.5 μm.

FIGURE 9. Transverse section through the intestine of *C. germanica* showing some of the different cell inclusions, including the electron-transparent inclusion. IN = inclusion, LU = lumen, SII = stage II inclusion. Bar is 0.5 μm.

FIGURE 10. Transverse section through the intestine of *C. germanica* showing some of the different cell inclusions. LU = lumen, MV = microvilli, N = nucleus, RER = rough endoplasmic reticulum, SI–II = Stage I–II inclusion, SIII = Stage III inclusion. Bar is 0.5 μm.

FIGURE 11. Transverse section of the intestine of *C. germanica* showing lead deposits, in a variety of cell inclusions, from the acid phosphatase reaction, not counterstained. I = intestine, LU = lumen. Bar is 0.5 μm.

approximately 6 μm, or twice the length, in the bulb region. Thus, *C. germanica* can exert more pressure on potential food.

The esophagus and intestine are separated by several cells forming a valve-like structure. These cells are typical protein-synthesizing cells.

In contrast to *Diplolaimella* sp., six cells encircled the intestinal lumen of *C. germanica* in any cross-section. Only one cell type was distinguishable with no regional specialization along the length of the intestine.

All intestinal cells have few, short, irregularly-spaced microvilli. The cells show transmembrane activity, possibly endocytosis, as indicated by small invaginations of the cell membrane that were coated on their outer leaflets with fibrillar or fuzzy material that varies in thickness and extends from the outer leaflet of the microvillar membrane into the lumen.

All intestinal cells appeared to have a potential for protein synthesis; each has typical protein-synthesizing features: extensive rough endoplasmic reticulum with distended cisternae, scattered Golgi complexes, and a prominent nucleus containing finely diffuse chromosomal material bounded by a nuclear membrane studded with ribosomes. This high potential degree of synthetic activity contrasts with the majority of the intestinal cells of *Diplolaimella* sp., which are absorptive.

A variety of membrane-bounded cell inclusions were randomly distributed in the intestinal cells throughout the length of the intestine. It is impossible to tell, however, from ultrastructure alone, if these inclusions represent different stages in the processing of digestive material. It is possible, however, to reconstruct a probable chronological development of these inclusions.

The largest sized inclusion body, Stage I (my terminology), contains small vesicular-shaped profiles and some membrane-like materials. Stage II is characterized by whorls of lamellae (Fig. 9). Transition stages between I and II are common (Fig. 10). Stage III is characterized by several large, dense, spherical granules enclosed by a single membrane (Fig. 10). The last stage, Stage IV, results from the possible coalescing of these granules to form inclusions which are sometimes uniformly very dense and sometimes grainy (Fig. 9). At times these inclusions are not fully infiltrated by the plastic embedding material. Stage IV appears similar to the lysosomal inclusions (pigment granules) found in *Diplolaimella* sp. These inclusions are sometimes observed in the process of being extruded into the gut lumen, thereby representing the final stage in the development of these inclusions. Another possibility is that these inclusions represent separate stages in two different processes. The Stage I inclusion is similar to an autophagosome and may be digesting worn-out cell parts. Stage II may be the residual body of this process. Or, Stage II may include the breakdown of cell membranes and may therefore function in cell membrane conservation. The Stages III and IV would still represent steps in the lysosomal digestion of food molecules.

Several other types of cytoplasmic inclusions have occasionally been observed in the intestinal cells: multivesicular bodies and vacuolar inclusions bodies are often associated with a golgi complex. Because the latter type of inclusion and some, but not all, of the stage IV inclusions are acid-phosphatase positive they can be considered lysosomes (Fig. 11).

There were occasional dying intestinal cells being sloughed off into the lumen throughout the length of the intestine. The cytoplasm of each dying cell exhibits

a gradient of density, in contrast to that of typical intestinal cells in *C. germanica,* with the least dense region of the cell protruding furthest into the intestinal lumen. This region consists of some microtubules and membrane-like material scattered throughout an electron-transparent matrix. The region of the cell closer to the cell's basal lamina contains a cytoplasm of nearly normal density with intact organelles or, in the case of cells in advanced stages of dying, with swollen mitochondria, a few distended cell inclusions, and short strips of rough endoplasmic reticulum. Significantly, there are no secretory products in these cells. This phenomenon may represent a type of holocrine secretion whereby the cell dies immediately after secreting its packaged enzymes into the lumen.

Ingestion and digestion of foods

Digestion in Diplolaimella *sp.* Reconstruction of the major events of digestion was deduced by following the natural food as it was broken down and by studying the digestive tracts of specimens in different nutritional states.

Food was generally ingested as whole, intact cells by the pumping action of the esophagus. The dorsal esophageal gland secretions coat the food when it enters the buccal cavity and then the subventral esophageal gland secretions coat the food cells as they move down into the esophageal lumen. There was no histochemical evidence that these secretions were enzymatic in nature, although these gland cells have been shown to secrete hydrolytic enzymes in other species (*e.g.,* Lee, 1965). Tests for esterase and arylamidase activities were negative in this region. These glands may be secreting components of one or more digestive enzyme which only become activated when mixed together in the lumen or on the food. The food may be further coated by the secretions from the cells of the esophageal-intestinal valve, although these cells were also negative for hydrolytic enzymes.

Morphological evidence for the initial chemical breakdown of the food was found in the intestinal lumen. Both intact and partially broken down food cells were distributed throughout the lumen (Figs. 12, 13). Esterases are involved in the initial stage of digestion, as indicated by a positive reaction for nonspecific esterase activity along the entire intestinal lumen.

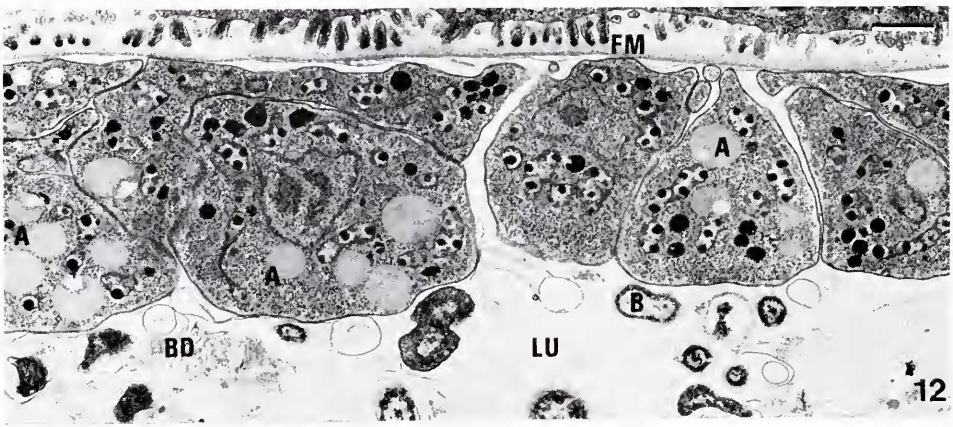
The esterases and other enzymes involved in luminal digestion may possibly be secreted by the esophageal gland cells, the valve cells, and the anterior secretory cells of the intestine. Luminal digestion may be the rate-limiting step in the digestive process, because well-fed nematodes subsequently deprived of food for four days still had intestines full of undigested food cells. This might indicate low concentrations of enzymes in the lumen.

At the same time that food enters the intestinal lumen, the intestinal cells, possibly the anterior secretory cells, secrete material for the fibrillar matrix. This matrix becomes sparse in starving animals, in contrast with well-fed specimens, which have a very dense matrix with homogeneously finely fibrillar material.

Well-fed specimens also had vesicles sprinkled throughout the anterior fibrillar matrix and along the length of the intestinal lumen, suggesting that the production of the matrix is coincidental with the ingestion of food.

Once the food has been broken into molecular size in the intestinal lumen, it presumably passes through the fibrillar matrix. This is suggested by the complete absence of detectable food particles in the matrix.

The presence of large numbers of acid phosphatase-positive lysosomes in the intestinal cells indicates that there is an intracellular phase of digestion. This phase probably begins in the less electron-dense cell inclusions and ends in the more electron-dense lysosomes. There are greater numbers of the former type of cell inclusion in well-fed specimens compared to specimens on diets of low-density food: the latter have from 0 to 4 of these inclusions and the former have about 15 to 40 inclusions in transverse sections. Further evidence that these inclusions contain food molecules comes from the fact that specimens raised on bacterial diets had intracellular inclusions filled with homogeneous material (Fig. 3) and those raised on algal diets had inclusions filled with fine grainy material.



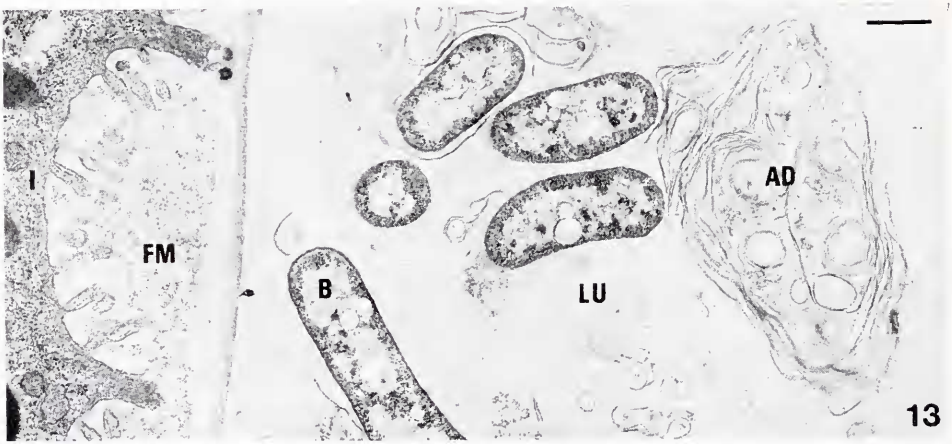


FIGURE 12. Longitudinal section of the intestine of *Diplolaimella* sp. showing amoebae "resting" on the fibrillar matrix. There are undigested and partially digested bacteria nearby. A = amoeba, B = bacterial cell, BD = bacterial cell debris, FM = fibrillar matrix, LU = lumen. Bar is 0.5 μm.

FIGURE 13. Longitudinal section of the intestine of *Diplolaimella* sp. showing the remains of *Dunaliella* sp. and several intact bacteria. AD = algal cell debris, B = bacterial cell; FM = fibrillar matrix, I = intestine, LU = lumen. Bar is 0.5 μm.

Similar grainy material was found in most of the Stage IV inclusions of *C. germanica* and may be characteristic of algal feeding.

Well-fed specimens had large numbers of glycogen particles surrounding both types of cell inclusions, although the lysosomes are usually closer to the luminal border of the cell and thus closer to the edge of the glycogen-containing area of the cell. This suggests that glycogen may be an end product of intracellular digestion. Undigested food debris collects in the posterior portion of the lumen, where it is periodically expelled through the rectum.

A protozoan with pseudopods, an amoeba, was found in the intestinal lumen of *Diplolaimella* sp. (Fig. 12).

Its role in digestion is unknown. Amoebae always occurred in the lumen wherever there was food and were absent from luminal areas devoid of food cells or food debris. In well-fed adults they occurred in numbers estimated at 50 to 100 individuals per worm. The amoebae appeared to be unaffected by the enzymes present in the lumen that breakdown the food cells: no partially digested remains were found in the lumen.

Their morphology indicated that they were similar to other intestinal amoebae and probably actively absorb and digest food.

Digestion in Chromadorina germanica. In contrast to *Diplolaimella* sp., *C. germanica* has a set of three teeth to puncture food cells and ingest their contents. A second set of teeth probably macerate the food further and may prevent particles beyond a certain size from entering the esophagus. Thus, digestion begins with the mechanical breakdown of food in the buccal cavity. Cell envelope fragments were never observed in the gut, so that only cellular contents and not cell envelopes must be sucked down through the esophagus. No enzymatic reactions were detected in the esophageal gland cells, suggesting that the action of these cells in secreting enzyme precursors is similar in both species. The food particles continue to the intestinal lumen. Here, they are further broken down by esterases and possibly by other enzymes. Then food molecules are absorbed by the intestinal cells, presumably by endocytosis. Digestion then proceeds in the various cell inclusions, probably begininning in the Stage I or Stage III inclusions. The final stage of digestion occurs in the Stage IV inclusion. The large amounts of secretory products found in the intestinal cells are primarily used in the lysosomal digestion of food. Digested food is stored as glycogen and, rarely, as lipid.

Results of feeding on different foods

Various species of algae and bacteria were fed to the two species of nematodes including two species of bacteria, four species of chlorophytes, and two species of diatoms. The bacterial cells were smallest, being about 2.2 μm in length. The chlorophytes ranged approximately from 2.2 to 4.9 μm in diameter or length. The diatoms were the largest at more than 10 μm long.

C. germanica survived on diets of the diatoms and three of the chlorophytes: *Dunaliella* sp., *D. salina,* and *Chlorococcum* sp.; but it was unable to survive on *Nanochloris* sp. and the bacterial species tested. *Diplolaimella* sp. was able to survive on the bacteria and chlorophytes but eventually died on diets of the diatoms. Since the cultures of both worms were contaminated with bacteria, starvation was a lingering process for *Diplolaimella* sp. It was probably able to use this alternative food source.

These results were borne out by examination of the guts of specimens fed various diets. The species of bacteria, D5-2 and A5-6, were found in the intestinal lumen of *Diplolaimella* sp. as both intact and partially digested cells (Fig. 11). No matter what else was offered to *Diplolaimella* sp., bacteria were always observed in the intestinal lumen of this animal (Fig. 13). On the other hand, bacteria were never identified in the intestinal lumen of *C. germanica*. Intestinal cells of specimens exposed to bacteria for periods longer than 2 hr wre in various stages of dying.

Undigested and partially digested *Nanochloris* sp. cells were easily identified in the intestinal lumen of *Diplolaimella* sp. Intact or partially digested algal cells were never observed in the gut of *C. germanica*. As in the case of bacterial feeding, the intestinal cells of specimens offered a diet of *Nanochloris* sp. appeared to be undergoing cell death. Remnants of *Chlorococcum* sp., particularly the distinctive starch grains, were found in the intestinal lumina of both nematode species. Broken cell fragments of *Dunaliella salina* occurred in the lumen of *Diplolaimella* sp., whereas both intact cells and cell fragments (Fig. 13) of *Dunaliella* sp. were found in the lumen of this nematode. Remains of neither *Dunaliella* species were found in *C. germanica,* although the intestinal cells appeared normal and were not all dying.

Cell fragments of *Amphora acutioscula,* a diatom, were found in the lumen of *C. germanica*. No cellular debris was found in the lumen of *Diplolaimella* sp. on this diet. Those specimens that did survive for a time on this diet had bacteria in the lumen. No recognizable fragments of the diatom *Cylindrotheca closterium* were found in the lumen of either nematode species, although the intestinal cells of *C. germanica* appeared normal—an indication that the food was being digested.

Discussion

The nematode species studied here have different methods of ingestion and digestion which are intimately related to their respective diets. *Diplolaimella* sp., due to its lack of buccal armature, ingests food solely by the sucking action of the esophagus. Its diet is limited to whole cells somewhat less than 3.9 μm in diameter, which include bacteria and the smaller chlorophytes, and cell fragments from cells greater than 3.9 μm in diameter, which include the thin-walled chlorophytes. This nematode is not anatomically equipped to exert enough pressure on the thicker, more rigid diatom wall to remove diatom cellular contents. The cell size limitation is actually not a direct result of the buccal capsule size, which is only about 1 μm in diameter. More likely, the cell size is a function of the diameter of the temporarily widened buccal capsule and esophagus during ingestion. The *Diplolaimella* sp. digestive tract, with the major portion of digestion occurring in the lumen, is adapted for processing whole bacterial and chlorophyte cells and chlorophyte cell fragments. It is interesting to note, in this regard, that *Monhystera denticulata,* a species closely related to *Diplolaimella* sp., produces β-glucuronidase in the intestine (Jennings and Deutsch, 1975). *Chromadorina germanica* does not produce this enzyme. This enzyme is probably utilized in hydrolyzing the cell envelope of ingested food cells because it is able to attack the glycan or polysaccharide portion of peptidoglycan (Ghuysen, Tripper and Strominger, 1966), a

major constitutent of the bacterial cell envelope, and the uronic acid residues composing the mucilages forming many algal cells walls (O'Colla, 1962).

In contrast, the buccal capsule armature of *C. germanica* enables it to ingest algal cell contents. It seems unable to ingest food cells less than 2.2 μm in diameter, probably because the pads and knobs forming the mouth are unable to hold and position these small food cells for piercing by the teeth. Cells less than 2.9 μm, the oral diameter, may either bounce off the teeth during attempts at ingestion or be filtered out by the esophageal teeth, the onchia, during ingestion. *C. germanica* also has a larger esophageal bulb which enables it to exert more suction on the diatom cell contents. Its digestive tract, therefore, is adapted for processing small algal food particles denuded of their cell envelopes.

The function of the nematode esophageal-intestinal valve has generally been thought to be, as its name implies, valvular (*e.g.*, Chitwood and Chitwood, 1950; Bird, 1971). In *Diplolaimella* sp., the circular muscle fibers between the esophagus and valve may function as a valve, but the position of the radiating muscle fibers of the valve itself suggest that this part of the valve, like the esophagus, pumps food through its lumen into the intestine. The protein synthesizing cells of the valve suggest a protein secretory role for these cells, and they may be secreting digestive enzymes involved in the digestive process in the intestinal lumen. This secretory function was also pointed out by de Man (1880) when he described a closely related species, *Monhystera vulgaris*, and observed a "cone-like group of gland cells" between the esophagus and the beginning of the intestine. In *C. germanica*, the valve is less complex and lacks any muscle fibers. Its ultrastructure also suggests that it may be secreting digestive enzymes, as well as acting as a valve structure.

The fibrillar matrix of *Diplolaimella* sp. has not been found in any other nematodes, including free-living and parasitic forms. This matrix may be acting as a molecular sieve, allowing only individual food molecules to pass into its regions. Although the present study did not test this idea, the concept of a molecular sieve is supported by evidence showing that the interface zone of the matrix contains cuticular proteins or cuticular protein-like molecules. If this zone is like a cuticle in chemical composition, then it would certainly limit the size of substances passing through it. The fibrils of the inner matrix zone may function in a type of contact digestion (Ugolev, 1965) in which food molecules may become adsorbed onto the fibrils, where enzymatic activity may then further hydrolyze them. This may be the reason for this matrix zone increasing in density with increased ingestion of food. The matrix may also be similar in function to the periotrophic membrane found in the midgut of certain insects (Smith, 1968) and crustaceans (Sheader and Evans, 1975) where it is believed to protect the intestinal cells from abrasion by hard particulate food. The periotrophic membrane encloses the food being digested and breaks up in the rectum to pass out, as fragments, with the feces (Jennings, 1972). In *Diplolaimella* sp., the fibrillar matrix was never observed to enclose the food, food was never seen embedded in it, nor did it occur with the undigested food debris. Nevertheless, the fibrillar matrix may function, in part, to protect the microvilli from abrasion by the relatively large food particles, in addition to its probable role of molecular sieve and substrate site for contact digestion.

The microvilli found in *Diplolaimella* sp. are different than those occurring in

the intestinal cells of other nematodes. The ultrastructure of these microvilli, whereby the electron-transparent material in the microvillus is bounded by a continuation of the endoplasmic reticulum, appears to be unique. This cisterna may be involved in the transport of food molecules from the microvillus to the lysosome, particularly since the microvillus swells in response to high levels of food being digested in the lumen.

Most nematodes have both lipid and glycogen storage inclusions in their intestinal cells (*e.g.*, Bird, 1971). The lack of lipid in *Diplolaimella* sp. and its low amount, relative to glycogen, in *C. germanica* may be an adaptation to short-term storage of food (Calow and Jennings, 1974).

The amoeba found in the *Diplolaimella* sp. intestine is probably ingesting some of the food of this nematode. It is possible that the amoeba is secreting enzymes that may be used by the nematode in luminal digestion or that the amoeba is excreting partially digested food that the nematode then utilizes. Any studies measuring food utilization by *Diplolaimella* sp., such as those for other free-living nematodes (Duncan, Schierner and Klekowski; 1974; Marchant and Nicholas, 1974; Nicholas, Grassia and Viswanathan, 1974), would have to consider that not all the food ingested by the worm is available for digestion.

Although one type of cell is present in the intestine of *C. germanica,* this cell probably goes through different phases of activity at different stages of its life. In a probable scheme, these cells may first be absorptive and digest food within their lysosomes. Older cells then become secretory and package digestive enzymes which are eventually released into the lumen. Then the cell dies. A similar cycle of growth of the intestinal cells described here does occur in a marine nematode, *Pontonema vulgaris,* (Jennings and Colam, 1970) where the cells are first absorptive and then, when they have aged, undergo a period of merocrine secretion.

A major difference between digestion in these two nematodes is in the emphasis on either luminal or lysosomal digestion. *C. germanica* has relatively fewer luminal stages to its digestion of food, as the major part of digestion occurs intracellularly by virtue of the elaborate set of cell inclusions and lysosomes described here. *Diplolaimella* sp. relies much more on luminal digestion, with only the final stages of digestion occurring intracellularly.

There may be an absolute limit on the number of different digestive enzymes one cell is capable of synthesizing. Thus, *C. germanica,* with its emphasis on lysosomal digestion, probably secretes fewer species of enzymes. To optimize its digestion, *C. germanica* must have a fairly narrow diet which would present the intestinal cells with fewer different molecules to hydrolyze, as each food cell has its own unique set of macromolecules. *Diplolaimella* sp., with its emphasis on luminal digestion, may be better adapted to digesting a greater variety of foods. Its few secretory cells may actually be capable of synthesizing a greater number of different enzymes. Its diet, compared to *C. germanica,* is broader, because it feeds on both algae and bacteria.

The phenomenon of selective feeding is shown here to be a function of the size of the food and also, for *Diplolaimella* sp., the type of cell envelope. As a result, these two species have the potential to overlap in their utilization of food resources when chlorophytes of a size utilizable by both are available but do not compete for diatoms and bacteria. Other studies also show that *C. germanica* prefers diatoms

and *Diplolaimella* sp. prefers bacteria (Tietjen and Lee, 1977). Selective feeding, then, may be an adaptive method for optimizing digestion—extracting the maximum usable molecules from the ingested food. In this way, a large number of nematode species in a benthic area could be avoiding interspecific competition while exploiting, as a taxon, a large diverse food source.

I wish to thank Drs. John J. Lee, Joseph Osinchak, Patricia Dudley, and J. B. Jennings for their advice and encouragement throughout this work. I especially wish to thank Dr. J. B. Jennings for first interesting me in the study of digestive physiology.

Summary

1. A comparative study has been made of gut ultrastructure, ingestion and digestion of a variety of foods by two salt marsh nematodes: *Chromadorina germanica* and *Diplolaimella* sp.

2. *Diplolaimella* sp. has a small, unarmoured buccal cavity. It has a complex esophageal-intestinal valve that consists of muscle and gland-like cells. The intestinal cells are of two types: the anterior four cells are secretory and the remaining cells are absorptive. There are unusual microvilli lining the luminal borders of these cells that contain a cisterna along the central axis that is continuous with the cisterna of the endoplasmic reticulm. An unusual, structurally complex, fibrillar matrix lines the luminal cell borders. An amoeba is found residing in the intestinal lumen wherever food is present.

3. *Chromadorina germanica* has a larger buccal cavity with two sets of three teeth. The intestine consists of secretory cells that are periodically sloughed off into the intestinal lumen. These cells have a variety of cell inclusions.

4. The process of digestion is described for each species. *Diplolaimella* sp. ingests whole cells or large cell fragments. Digestion begins chemically in the intestinal lumen with secretions from the esophageal gland cells and esophageal-intestinal valve cells. It is completed intracellularly in the intestinal cell pigment granules, which are demonstrated to be lysosomes.

5. *C. germanica* pierces its food and ingests the cell contents. Digestion continues in the intestinal lumen with secretions from the esophageal gland cells and the intestinal cells. Digestion is completed intracellularly in a variety of lysosomes.

6. *Diplolaimella* sp. ingests whole bacteria and small chlorophytes and large cell fragments of the larger chlorophytes. It does not ingest diatoms. *C. germanica* ingests the cell contents of diatoms and large chlorophytes but does not ingest small chlorophytes or bacteria.

LITERATURE CITED

Barka, T., and P. J. Anderson, 1962. Histochemical methods for acid phosphatase using hexazonium pararosanalin as coupler. *J. Histochem. Cytochem.*, **10**: 741–749.

Bird, A. F., 1971. *The structure of nematodes.* Academic Press, New York, 318 pp.

Burstone, M. S., and J. E. Folk, 1956. Histochemical demonstration of aminopeptidase. *J. Histochem. Cytochem.*, **4**: 217–226.

Calow, P., and J. B. Jennings, 1974. Calorific values in the Phylum Platyhelminthes: the relationship between potential energy, mode of life and the evolution of entoparasitism. *Biol. Bull.*, **147**: 81–94.

Chitwood, B. G., and M. B. Chitwood, 1950. *An introduction to nematology.* University Park Press, Baltimore, 213 pp.

Colam, J. B., 1971a. Studies on gut ultrastructure and digestive physiology in *Rhabdias bufonis* and *R. sphaerocephala* (Nematoda: Rhabditida). *Parasitology*, **62**: 247–258.

Colam, J. B., 1971b. Studies on gut ultrastructure and digestive physiology in *Cyathostoma lari* (Nematoda; Strongylida). *Parasitology*, **62**: 273–283.

Coull, B. C., 1970. Shallow water meiobenthos of the Bermuda platform. *Oecologia*, **4**: 325–357.

Duncan, A. F. P. Schiemer, and R. J. Klekowski, 1974. A preliminary study of feeding rates on bacterial food by adult females of a benthic nematode, *Plectus palustris* De Man 1880. *Pol. Arch. Hydrobiol.*, **21**: 249–258.

Fenchel, T., 1969. The ecology of marine microbenthos. IV. Structure and function of the benthic ecosystem, its chemical and physical factors and the microfauna communities with special reference to the ciliated protozoa. *Ophelia*, **6**: 1–182.

Gerlach, S. A., and M. Schrage, 1971. Life cycles in marine meiobenthos. Experiments at various temperatures with *Monhystera disjuncta* and *Theristus pertenuis* (Nematoda). *Mar. Biol.*, **9**: 274–280.

Gerlach, S. A., and M. Schrage, 1972. Life cycles at low temperatures in some free-living marine nematodes. *Veroeff. Inst. Meeresforsch. Bremerhaven*, **14**: 5–11.

Ghuysen, J. M., D. J. Tripper, and J. L. Strominger, 1966. Enzymes that degrade bacterial cell walls. Pages 685–699 in Elizabeth F. Neufeld and Victor Ginsberg, Eds., *Methods in Enzymology: Complex Carbohydrates*, Vol. 8. Academic Press, New York.

Holt, S. J., 1958. Studies in enzyme histochemistry. *Proc. R. Soc. Lond. B. Biol. Sci.*, **148**: 465–532.

Hopper, B. E., J. W. Fell, and R. C. Cefalu, 1973. Effect of temperature on life cycles of nematodes associated with mangrove (*Rhizophora mangle*) detrital system. *Mar. Biol.*, **23**: 293–296.

Jennings, J. B., 1972. *Feeding, digestion and assimilation in animals.* Macmillan and Co., New York, 244 pp.

Jennings, J. B., and J. B. Colam, 1970. Gut structure, digestive physiology and food storage in *Pontonema vulgaris* (Nematoda: Enoplida). *J. Zool. (Lond.)*, **161**: 211–221.

Jennings, J. B., and A. Deutsch, 1975. The occurrence and possible adaptive significance of β-glucuronidase and arylamidase ("leucine aminopeptidase") activity in two species of marine nematodes. *Comp. Biochem. Physiol.*, **52A**: 611–614.

Lee, D. L., 1965. *The physiology of nematodes.* W. H. Freeman and Co., San Francisco, 154 pp.

Lee, D. L., and H. J. Atkinson, 1976. *Physiology of nematodes.* Macmillan Press Ltd., London, 218 pp.

Lee, J. J., M. E. McEnery, E. M. Kennedy, and H. Rubin, 1975. A nutritional analysis of a sublittoral diatom assemblage epiphytic on *Enteromorpha* from a Long Island salt marsh. *J. Phycol.*, **11**: 14–49.

Lee, J. J., J. H. Tietjen, R. J. Stone, W. A. Muller, J. Rullman, and M. McEnery, 1970. The cultivation and physiological ecology of members of salt marsh epiphytic communities. *Helgol. Wiss. Meersunters.*, **20**: 136–156.

Man, J. G., de, 1880. *Tijdschr. ned. dierk. Ver.*, **5**: 1–104.

Marchant, R., and W. L. Nicholas, 1974. An energy budget for the free-living nematode *Pelodera* (Rhabditidae). *Oecologia*, **16**: 237–252.

McIntyre, A. D., 1969. Ecology of marine meiobenthos. *Biol. Rev. Camb. Philos. Soc.*, **44**: 245–290.

Meyers, S. P., B. E. Hopper, and R. Cefalu, 1970. Ecological investigations of the marine nematode *Metoncholaimus scissus*. *Mar. Biol.*, **4**: 43–47.

Nicholas, W. L., A. Grassia, and S. Viswanathan, 1974. The efficiency with which *Caenorhabditis briggsae* (Rhabditidae) feeds on the bacterium, *Escherichia coli*. *Nematologica*, **19**: 411–420.

O'Colla, P., 1962. Mucilages. Pages 337–356 in R. A. Lewin, Ed., *Physiology and biochemistry of algae,* Academic Press, New York.

Ott, J., and F. Schiemer, 1973. Respiration and anaerobiosis of free-living nematodes from marine and limnic sediments. *Neth. J. Sea Res.,* **7**: 233–243.

Pamatmat, M. M., 1968. Ecology and metabolism of a benthic community on an intertidal sandflat. *Int. Revue Gesamten Hydrobiol.,* **53**: 211–298.

Quintarelli, G., M. Bellocci, and R. Geremia, 1973. On phosphotungstic acid staining IV. Selectivity of the staining reaction. *J. Histochem. Cytochem.,* **21**: 155–160.

Sato, T., 1967. A modified method for lead staining of thin sections. *J. Electron Microsc.,* **16**: 133–136.

Schiemer, F., and A. Duncan, 1974. The oxygen consumption of a freshwater benthic nematode, *Tobrilus gracilis* (*Bastian*). *Oecologia,* **15**: 121–126.

Sheader, M., and F. Evans, 1975. Feeding and gut structure of *Parathemisto gaudichaudi* (Guerin) (Amphipoda, Hyperiidea). *J. Mar. Biol. Assoc. U. K.,* **55**: 641–656.

Smith, D. S., 1968. *Insect cells: their structure and function.* Oliver and Boyd Ltd., Edinburgh, 372 pp.

Spurr, A. R., 1969. A low-viscosity epoxy resin embedding medium for electron microscopy. *J. Ultrastruct. Res.,* **26**: 31–43.

Tietjen, J. H., 1969. The ecology of shallow water meiofauna in two New England estuaries. *Oecologia,* **2**: 251–291.

Tietjen, J. H., 1971. Ecology and distribution of deep-sea meiobenthos off North Carolina. *Deep-Sea Res.,* **18**: 941–957.

Tietjen, J. H., and J. J. Lee, 1972. Life cycles of marine nematodes: influence of temperature and salinity on the development of *Monhystera denticulata Timm. Oecologia,* **10**: 167–176.

Tietjen, J. H., and J. J. Lee, 1973. Life history and feeding habits of the marine nematode, *Chromadora macrolaimoides* Steiner. *Oecologia,* **11**: 303–314.

Tietjen, J. H., and J. J. Lee, 1977. Feeding behavior of marine nematodes. Pages 21–35 in B.C. Coull, Ed., *Ecology of marine benthos.* University of South Carolina Press, Columbia.

Tietjen, J. H., J. J. Lee, J. Rullman, A. Greengart, and J. Trompeter, 1970. Gnotobiotic culture and physiological ecology of the marine nematode *Rhabditis marina* Bastian. *Limnol. Oceanogr.,* **15**: 535–543.

Ugolev, A. M., 1965. Membrane (contact) digestion. *Physiol. Rev.,* **45**: 555–595.

Ward, A. R., 1973. Studies on the sublittoral free-living nematodes of Liverpool Bay. I. The structure and distribution of the nematode populations. *Mar. Biol.,* **22**: 53–66.

Warwick, R. M., 1971. Nematode associations in the Exe estuary. *J. Mar. Biol. Assoc. U.K.,* **51**: 439–454.

Warwick, R. M., and J. B. Buchanan, 1970. The meiofauna off the coast of Northumberland I. The structure of the nematode population. *J. Mar. Biol. Assoc. U.K.,* **50**: 129–146.

Wieser, W., 1953. Die Beziehung zwischen Mundhohlengestalt, Ernahrungswiese und Vorkommen bei freillebenden marienen Nematoden. *Ark. Zool.,* **4**: 439–484.

Wieser, W., 1960. Benthic studies in Buzzards Bay. II. The meiofauna. *Limnol. Oceanogr.* **5**: 121–137.

Wieser, W., and F. Schiemer, 1977. The ecophysiology of some marine nematodes from Bermuda: seasonal aspects. *J. Exp. Mar. Biol. Ecol.,* **26**: 97–106.